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Reductive Dehalogenation of Trichloroethene Vapors in an Anaerobic Biotrickling Filter

SUDEEP C. POPAT[†] AND MARC A. DESHUSSES*[,‡]

Department of Chemical & Environmental Engineering, University of California, Riverside, California 92521, and Department of Civil & Environmental Engineering, Duke University, Durham, North Carolina 27708

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Until now, it has not been possible to use biofiltration to treat trichloroethene (TCE) from waste gases generated by soil vapor extraction or dual-phase extraction at remediation sites because aerobic biodegradation of TCE is possible only via cometabolism, which is difficult to engineer on a large scale. This study looks at the possibility of conducting anaerobic gas-phase biotreatment of TCE vapors. The vision is that nitrogen sparging could be substituted for air sparging, resulting in TCE contaminated oxygen-free gas streams which require treatment. A lab-scale anaerobic biotrickling filter inoculated with a mixed culture containing multiple *Dehalococcoides* strains was used for the proof of concept. TCE vapors were removed via reductive dechlorination and converted to ethene, *cis*-1,2-dichloroethene (*cis*-DCE), and vinyl chloride (VC). Sodium lactate, a fermentable substrate, was provided to the reactor through the recirculating liquid as a source of hydrogen, the electron donor for *Dehalococcoides* strains. The biotrickling filter was able to remove >90% TCE at loadings of up to 4 g m_{bed}^{-3} h^{-1} and sustained performance for over 200 days. The distribution of the intermediates of TCE biological reduction was found to be affected by the pH of the recirculating liquid. At pH 8.3, the primary accumulating product was *cis*-DCE (~92% of the TCE removed); while at pH 6.85−6.9, conversion to ethene, the intended end product, was 50−67% of the TCE removed. Kinetic determinations using batch biotrickling filter operation showed that VC reduction and not *cis*-DCE reduction was the slowest step. Overall, the study shows that sustained anaerobic biotreatment of TCE vapors in biotrickling filters is possible.

Introduction

Trichloroethene (TCE) is a relatively persistent vadose zone and groundwater pollutant that is toxic and classified as a likely human carcinogen (*1, 2*). In the United States, there are thousands of TCE contaminated sites that pose enough risk that remediation is necessary. Notably, an estimated 852 out of 1430 National Priorities List sites identified by the Environmental Protection Agency are contaminated with TCE (*3*). Techniques used for removing TCE from contaminated soil and groundwater include soil vapor extraction (SVE) in combination with air sparging and dual-phase extraction (DPE) (*4*). However, these techniques result in a waste gas stream that needs further treatment. Several methods such as incineration, catalytic oxidation, and adsorption onto activated carbon are currently being used to control TCE vapors thus generated (*5*). These methods are expensive because of high operational and energy costs, and they are nonsustainable because of the need for further treatment or disposal of the spent activated carbon. Biofiltration is an attractive technology for treating volatile organic compounds because it is often cost-effective and environmentally friendly (*6*). Thus, the development of suitable biological methods for the treatment of TCE vapors is desirable.

Moderate removal of TCE in lab-scale aerobic bioreactors has been observed only when fed with a primary substrate such as methane, toluene, or phenol, leading to fortuitous degradation (i.e., cometabolism) of TCE by the primary substrate-specific oxygenase enzymes (*7, 8*). Even so, this has not found practical application because of the frequent loss of performance due to the formation of toxic intermediates, such as TCE epoxide, and competitive inhibition with the primary substrate (*9, 10*). Thus, it is necessary to exploit alternative microbial metabolic pathways to biologically remove TCE from waste gas streams.

In anaerobic environments, chlorinated solvents can be used as electron acceptors by microorganisms in the presence of a favorable electron donor (*11*). Under reducing conditions, anaerobic microorganisms from the *Dehalococcoides* genus reduce tetrachloroethene (PCE) or TCE while oxidizing hydrogen (*12*), leading to progressive formation and reduction of *cis*-1,2-dichloroethene (*cis*-DCE) and vinyl chloride (VC) to ethene. *Dehalococcoides ethenogenes* strain 195 was the first isolated microorganism able to reduce PCE all the way to ethene. However, it reduces VC only cometabolically in the presence of higher chlorinated ethenes and at slow rates (*13*). For a long time, VC was believed to be recalcitrant anaerobically, until He et al. (*14*) isolated the first bacterium, *Dehalococcoides* sp. strain BAV1 that grew primarily on VC, reducing it to ethene. Other *Dehalococcoides* strains that degrade PCE or TCE all the way to ethene have since been isolated (*15, 16*).

Dehalococcoides strains have been used in several in situ bioremediation applications through bioaugumentation with mixed cultures, with success in some cases (*17*) but failure in others (*18*). The most notable problem associated with lack of successful treatment is accumulation of *cis*-DCE or VC, as a result of unfavorable growth conditions for *Dehalococcoides* strains. However, it should be possible to treat oxygen-free waste gas streams containing TCE in anaerobic bioreactors in which mixed cultures of *Dehalococcoides* strains are grown and wherein the conditions for the growth of these microorganisms can easily and effectively be controlled. For TCE contaminated site remediation, nitrogen generators could be used to displace oxygen, resulting in the recovery of oxygen-free TCE contaminated gas streams that require treatment. Traditionally, nitrogen generation has been done using expensive cryogenic techniques, but now, with improved pressure swing adsorption and membrane separations, the cost of large-scale nitrogen generators has been dramatically reduced.

Thus, the objective of this study was to provide the proof of concept for the removal of TCE vapors in anaerobic gas-phase bioreactors and identify any challenges. A lab-scale anaerobic biotrickling filter fed with a TCE-laden nitrogen stream and inoculated with a commercially available mixed culture containing multiple *Dehalococcoides* strains was set

* Corresponding author phone: (919) 660-5480; fax: (919) 660-5219; e-mail: marc.deshusses@duke.edu.
[†] University of California.
[‡] Duke University.

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up and monitored for its performance. The distribution of dechlorination intermediates was characterized at different operating conditions in the bioreactor and biological reduction rate constants for TCE, *cis*-DCE, and VC were determined using a batch setup.

Materials and Methods

Culture Used. The seed culture containing *Dehalococcoides* strains used for this study was commercially available (SDC-9, Shaw Environmental, Inc., Lawrenceville, NJ). It contains at least two *Dehalococcoides* strains, a *Desulfitobacterium* strain, a *Desulfovibrio* strain, other fermenters, hydrogenotrophic methanogens, and possibly homoacetogens (see the Supporting Information for further details); however, it does not contain a significant amount of aceticlastic methanogens. The seed culture contained $\sim 10^8$ *Dehalococcoides* spp. cells mL^{-1} (determined by Shaw Environmental, Inc. using qPCR techniques) and was stored at 4 °C in oxygen-free vials no longer than 3 months before use.

Biotrickling Filter Setup and Operation. A schematic of the experimental setup is shown in Figure S1 (see the Supporting Information). The biotrickling filter was constructed from a clear PVC pipe 60 cm long and 10 cm in internal diameter (Harrington Plastics, Chino, CA). The packing material used for the bed was cattle bone Porcelite (CBP), a porous spherical ceramic with slow-release mineral nutrients incorporated (Aisin Takaoka Co., Ltd., Japan) (*19*). The packing material had an average diameter of particles of 3 mm. The active bed height was 30 cm, and thus, the bed volume was 2.4 L. The bed void volume was determined to be 42%.

The anaerobic waste gas stream was generated by sparging compressed nitrogen (99.97% pure, Airgas, Riverside, CA) through a 1 L bottle of water at room temperature first to humidify it and then by passing it through a 500 mL bottle containing a 40 mL vial filled with TCE (Fisher Chemical, Fairlawn, NJ). To achieve the desired concentrations (60−300 $mg\,m^{-3}$), the vial was closed with a cap and a septum through which different syringe needles (15−18 G) were inserted in the gas headspace to change TCE inlet concentrations. The inlet concentration was selected to be representative of a medium to low concentration of a possible SVE or DPE field remediation.

A constant liquid sump volume of 1 L was maintained at the bottom of the reactor and recirculated over the bed at the rate of 20 mL min^{-1} initially and at 50 mL min^{-1} after 39 days of operation. The bed was inoculated initially by adding 100 mL of SDC-9 culture to the sump. The mineral medium used in the biotrickling filters was modified RAM medium (Table S1 in the Supporting Information) (*20*) with sodium lactate (60% sodium lactate solution, Fisher Chemical, Fairlawn, NJ) added to a final concentration of 1.0−3.2 $g\,L^{-1}$ as the fermentable substrate. During the first 4 days of operation, the reactor was operated in a closed loop in terms of the liquid to maximize the cell adhesion to the packing. Sodium lactate was added to the liquid on day 0 (8.2 g) and day 3 (10.4 g) of operation. From day 5 onward, fresh mineral medium containing approximately 10 times the stoichiometric requirement of lactate for complete TCE dechlorination was fed to the reactor at the rate of 800 mL day^{-1}. The stoichiometric requirement was calculated neglecting the hydrogen that could result from the potential fermentation of acetate, a byproduct of lactate degradation. The decision to provide excess lactate was motivated by the presence of hydrogenotrophic methanogens and possibly homoacetogens in the culture that would compete with *Dehalococcoides* spp. for hydrogen. Lactate fermentation leads to a higher hydrogen yield compared to other fermentable substrates (e.g., methanol, acetate, propionate, butyrate, etc.), and hydrogenotrophic methanogenesis is especially expected even at low lactate concentrations (*21*). The mineral medium feed was kept at 4 °C to prevent lactate fermentation before feeding into the reactor. The stock mineral medium contained approximately 4 mg L^{-1} dissolved oxygen; this represented a negligible oxygen supply to the system.

Studies on the Effect of pH of Recirculation Liquid. The effect of recirculating liquid pH on the distribution of TCE reduction intermediates was investigated by changing the concentration of KH_2PO_4, K_2HPO_4, and $NaHCO_3$ in the mineral medium feed. These experiments were performed 91 days after the reactor startup. The bioreactor bed was reinoculated with 50 mL of SDC-9 culture, having the same concentration of *Dehalococcoides* cells as the initial inoculum, to ensure all members of the original consortium were still present, and the amount of lactate added was changed to 12.5 times the stoichiometric requirement for complete TCE dechlorination. Each pH was maintained for 6−7 days, and analyses performed on days 3, 4, 5, and 6 after the pH change were used to calculate average conversion.

Batch Biotrickling Filter. After 180 days, the biotrickling filter was modified to operate in a closed loop with respect to the gas (hereafter called batch mode) to evaluate the biological reduction rate kinetics for TCE, *cis*-DCE, and VC. Briefly, the gas outlet of the reactor was connected to the inlet port of the biotrickling filter. A 1 L Tedlar bag and a diaphragm pump (Air Dimensions Inc., Deerfield Beach, FL) were connected in the gas recycle loop to provide extra gas for sampling and the means for rapid recycling of gas, respectively (Figure S2 in the Supporting Information). The gas was recirculated at a rate 3.9 L min^{-1} (corresponding to an empty bed residence time (EBRT) of 39 s). At the beginning of each experiment, known amounts of each compound (TCE, DCE, VC) were individually spiked into the inlet port. To minimize the amount of pollutant partitioning to the liquid phase, the sump volume was reduced to 50 mL. The water content of the bed was estimated to be 10−20% by weighing the biotrickling filter and comparing it to the weight of a clean system. The experiments involved monitoring the decrease of each compound with time and fitting the data obtained after one hour (i.e., the time to reach gas−liquid pseudo steady state) to a first-order kinetic model. Multiple experiments starting with slightly different initial concentrations gave values of apparent rate constants within 5% of each other. Methane formation rates were used as an indication of hydrogen availability. Because SDC-9 contains only hydrogenotrophic methanogens, methane formation rates could be used as indication of hydrogen availability. For all experiments reported, the rate of methane production did not decrease, suggesting that there was sufficient hydrogen produced from the residual lactate in the sump liquid and in the bed dynamic holdup (data not shown).

Analysis. Gas-phase concentrations of TCE, *cis*-DCE, VC, ethene, and methane were quantified using an HP 5890 Series II gas chromatograph, fitted with a 30 m (0.32 mm internal diameter) GS-Q column (Agilent Technologies, Inc., Wilmington, DE) and a flame ionization detector. The detection limits for TCE, *cis*-DCE, VC, ethene, and methane were 2, 1, 1, 0.5, and 0.5 mg m^{-3}, respectively. Wherever needed, the liquid concentrations were calculated using the Henry's constants for each chlorinated compound in water reported by Gossett (*22*). The values of Henry's dimensionless constants at 25 °C are 0.392, 0.167, and 1.137 for TCE, *cis*-DCE, and VC, respectively. The effect of dissolved salts or lactate on Henry's constants was less than 10% (results not shown), and the above values were used throughout this study. Total organic carbon (TOC) content in the recirculating liquid was measured periodically on a TOC-5050 analyzer (Shimadzu Scientific Instruments, Columbia, MD). Redox potential (ORP) and dissolved oxygen (DO) in the recirculating liquid were measured periodically using an ORP sensor and

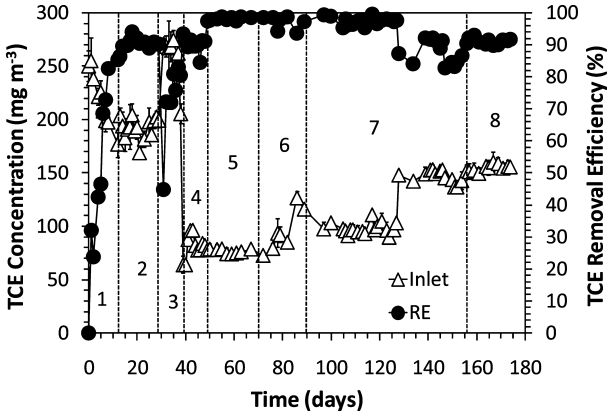

FIGURE 1. Continuous operation of the anaerobic biotrickling filter removing TCE. Different phases of treatment denoted by Arabic numerals are described in Table 1.

a DO sensor, respectively (Vernier Software & Technology, Beaverton, OR). The pH of the recirculating liquid was monitored regularly using an Accumet pH meter (Cole-Parmer Instrument Co., Vernon Hills, IL). Pressure drop across the biotrickling filter bed was measured periodically using a U-tube manometer.

Results and Discussion

Continuous Operation of the Biotrickling Filter. Figure 1 shows the performance of the anaerobic biotrickling filter removing TCE continuously for 180 days, while Table 1 lists the conditions during each experimental phase. There was a 13-day startup phase for the reactor during which the TCE removal efficiency (RE) of the reactor gradually increased to close to 90% at a gas EBRT of 3 min. Typical startup times for aerobic biotrickling filters are about one week, as observed with several model pollutants, for example toluene (*23*). On the basis of the 21.5 h doubling time reported for *Dehalococcoides* spp. in SDC-9 culture (*24*) and the TCE specific reduction rate for the culture determined in batch studies of 1.3×10^{-12} $mmol_{TCE}$ $cell_{DHC}^{-1}$ h^{-1} (*25*), it was estimated that the startup time would last about 5 days at the loadings imposed (Figure S3 in the Supporting Information). Removal of TCE by physical absorption into the trickling liquid was expected to be the major mechanism of removal for the first 4 days based on gas−liquid equilibrium calculations. Indeed, higher than expected removal was observed the first three days. Metabolites of TCE reductive dehalogenation were detected from day 2 onward indicating that some biological activity was already occurring in the reactor. Still, it took longer than expected to reach a steady removal of TCE, and the RE did not reach 100% as expected. Some kinetic limitations determined later in the study may have played a role.

From day 14 onward (phase 2), the biotrickling filter was operated at TCE inlet concentrations of 200 ± 20 mg m^{-3}, and a sustained RE of around 90% was observed. This corresponds to a TCE elimination capacity (EC) of 3−4 g m_{bed}^{-3} h^{-1}. A minor increase in pressure drop was also observed for the first time during operation (Table S2 in the Supporting Information), as a result of biomass growth. Subsequently, the objective was to determine the TCE EC at high loadings to evaluate the maximum capacity of the biotrickling filter, and thus, the TCE inlet concentration was increased on day 30 (phase 3). Following a brief low RE transient phase due to the failure of the lactate feed pump on day 30, the TCE RE reached a steady value of about 80%, with a maximum TCE elimination capacity of 4.4 g m_{bed}^{-3} h^{-1}.

During phases 4−8, the reactor was subjected to lower TCE loadings through either decrease in inlet concentration or increase in EBRT. This was done to observe the distribution of intermediates, which during the earlier phases showed that most of the TCE removed was converted to *cis*-DCE (~85%) and not ethene, the intended end product. Conversion to VC was low (<10% of TCE removed). This observation contrasts with reductive dehalogenation of TCE in microcosms with isolated *Dehalococcoides* strains which usually results in complete sequential conversion to ethene (*13, 15, 16*). The main reason for the difference is probably the continuous loading of TCE in the present biotrickling filter vs batch mode in microcosms. Although *cis*-DCE is less toxic than TCE (*26*), it is still regulated (*27*), whereas VC has been identified as a human carcinogen (*28*). *cis*-DCE was found to significantly partition into the liquid, due to its lower Henry's constant compared to TCE and VC (see values in Materials and Methods section). It was hypothesized that the slight accumulation of *cis*-DCE in the liquid (up to 220 mg m^{-3}) may be not only the result of slower reduction but also the low contact time of the liquid with bacteria attached to the packed bed. Hence, the liquid recirculation rate was increased 2.5 fold on day 39, but this did not significantly affect the distribution of metabolites. Longer gas EBRT also did not significantly shift the distribution of the intermediates (results not shown), and TCE RE throughout these phases remained around 90%.

Throughout the study, the ORP and DO of the recirculating liquid remained in the range of 90−110 mV and 0.7−0.8 mg L^{-1}, respectively, suggesting slightly oxidizing conditions. The reason why completely reducing conditions were not observed could be because the influent medium was not sparged with oxygen-free gas before feeding to remove oxygen. This introduced a minute amount of oxygen (compared to the total BOD load) which was deemed negligible. Amos et al. studied the effect of oxygen in microcosms containing the BDI consortium (a different commercially available TCE

TABLE 1. Operating Conditions during the Different Experimental Phases[a]

	phase	inlet TCE concentration (mg m^{-3})	EBRT (min)	pH of recirculating liquid
1	startup period	175−250	3	7.6−7.8
2	pseudo steady-state period	190 ± 20	3	7.7−7.8
3	higher TCE loading	270 ± 10	3	7.7−7.8
4	lower TCE loading, and faster liquid recirculation rate	85 ± 15	3	7.8−7.9
5	EBRT increased	80 ± 10	4.8	7.9
6	lactate concentration increased[b]	80−130	4.8	8.1−8.3
7	pH controlled studies[c]	90−150	4.8	6.6−8.3
8	steady state at optimum pH	145 ± 10	4.8	6.85
9	batch studies	variable	NA	6.8−7.0
10	steady-state period (Table S4 of the Supporting Information)	245−255	4.8	6.85−6.91

[a] See text for details. [b] Lactate concentration increased from 10× to 20× the stoichiometric requirement for complete TCE dechlorination. [c] Lactate concentration decreased from 20× to 12.5× for the rest of the study.

reducing culture). They showed that VC (and not *cis*-DCE) accumulated significantly in the presence of residual oxygen (*29*). Thus, the accumulation of *cis*-DCE reported here is probably not linked to the positive ORP. Nonetheless, further investigations of the effect of ORP and DO in TCE-degrading biotrickling filters are warranted, since this can be expected to be an important parameter. Further, all studies on the effect of oxygen on *Dehalococcoides* strains have been done in suspended cultures, but the effect on *Dehalococcoides* in a complex biofilm needs attention.

TOC analyses of the liquid revealed that only 30−60% of the organic carbon fed to the biotrickling filter as lactate was used, suggesting that the lactate feed rate could be optimized. The organic carbon utilization rate corresponds to an EC of 3−7 $g_C\ m_{bed}^{-3}\ h^{-1}$, which is a low value compared to VOC degrading gas-phase bioreactors (*30, 31*). Even so, it was feared that over time, the growth of fermentors would cause clogging problems in the biotrickling filter, especially considering the high density and low porosity of the ceramic packing compared to more open packing such as Pall rings or polyurethane foam cubes and high biomass yields for fermentation. Biotrickling filters subjected to high organic loadings have experienced clogging problems because of excess biomass growth (*30*). The low overall pressure drop observed (Table S2 in the Supporting Information) throughout the study indicates that this was not the case here. The slow biomass accumulation was most probably due to the relatively low organic loading compared to biotrickling filters that experience rapid plugging.

The continuous operation of the reactor for 180 days without any significant loss of activity is an important finding. The production of methane (up to 300 mg m^{-3}), which was observed throughout the operation of the biotrickling filter, did not seem to affect the rate of TCE reductive dehalogenation. Freeborn et al. (*21*) studied the activity of *Dehalococcoides* strains in mixed cultures with respect to different sources of hydrogen and found that, with the use of lactate, significantly higher hydrogen yields and methanogenesis were observed, but there was no detrimental effect of the activity of the methanogens on the rate of dechlorination. Even so, because methane is a potent greenhouse gas, further research should focus on optimizing the electron donor source in TCE-removing biotrickling filters to minimize methane production. Additionally, we did not detect volatile fatty acids (VFAs) in the gaseous effluent. Considering the low volatility of VFAs, the cumulative concentration of VFAs in the recirculating liquid was estimated to be no more than 1 g L^{-1}, which is not negligible in the overall lactate−carbon balance but remains far below the inhibitory level of 6 g L^{-1} for reductive dechlorination reported by Vainberg et al. (*25*).

Steady-State Performance. Figure 2 shows the TCE EC vs load for the biotrickling filter at steady-state operation. The biotrickling filter was able to remove >90% TCE at loadings up to 4 g $m_{bed}^{-3}\ h^{-1}$ at EBRT of 3 min. At higher TCE loadings, breakthrough was observed; loadings of over 5 g $m_{bed}^{-3}\ h^{-1}$ resulted in only about 80% removal of TCE. At an EBRT of 4.8 min, breakthrough was not observed at the loadings tested. To our knowledge, only two studies on biological gas-phase removal of TCE in aerobic bioreactors have reported a higher EC (Table S3 in the Supporting Information). Sun and Wood (*32*) observed a maximum TCE EC of 16.3 g $m_{bed}^{-3}\ h^{-1}$ in a fixed-film biofilter inoculated with a pure culture of *B. cepacia* G4, a mutant expressing the toluene *ortho*-monooxygenase enzyme constitutively and fed with glucose as the primary carbon substrate. This performance however was not sustained, and cell decay was observed within a day, probably due to inhibition by TCE epoxide. Kan and Deshusses (*33*) observed a maximum TCE EC of 28 g $m_{bioreactor}^{-3}\ h^{-1}$ in a foamed emulsion bioreactor using toluene as a primary substrate for TCE cometabolism.

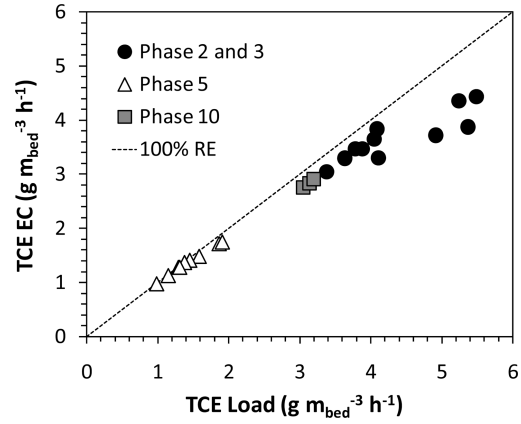

FIGURE 2. Pseudo steady-state TCE elimination capacity (EC) vs load for the anaerobic biotrickling filter. The EC is calculated as the mass of TCE removed hourly in the system divided by the volume of the bed.

However, the foam bioreactor is a relatively complex system, and both scale-up and maintaining optimum performance may be difficult.

Only one other study has looked at gas-phase removal of chlorinated solvents in bioreactors anaerobically. Schwarz et al. (*34*) reported an elimination capacity of 4.1 g $m_{bed}^{-3}\ h^{-1}$ for PCE after 100 days of operation of a biofilter packed with activated carbon, inoculated with anaerobic sludge, and fed with simulated biogas. Sucrose was used as the fermentable substrate for hydrogen production. The microbial culture was a mixed culture from anaerobic sludge from a facility contaminated with PCE. It is not known whether their bioreactor contained any *Dehalococcoides* strains. It is worthwhile to note that no biological reduction intermediates were observed and that the final product of dechlorination was also not identified. Adsorption onto the activated carbon bed may have played a role in the control of intermediates. The adsorption of PCE onto the packing media was studied, while that of TCE, *cis*-DCE, and VC was not determined.

Effect of pH on the Distribution of Intermediates. It was originally hypothesized that the low conversion of TCE to ethene could be due to hydrogen limitation. This could occur even when excess lactate was used because of hydrogen consumption by methanogens. Other researchers have observed *cis*-DCE accumulation during PCE treatment under electron donor limiting conditions (*35, 36*). Thus, the lactate feed was increased to 20 times the stoichiometric requirement (phase 6 in Figure 1 and Table 1). TCE conversion shifted to even more unfavorable values with 92% of the TCE removed accumulating as *cis*-DCE and only 4% conversion to ethene. It was noted that the pH of the recirculating medium increased from 7.9 to 8.3. Reductive dechlorination typically results in a decrease in pH through production of protons and chloride ions; however, accompanying hydrogen-consuming processes can drive the pH up by consumption of protons. The larger lactate load resulted in larger methane formation (data not shown), thus increasing the pH. Thereafter, the pH was controlled by adjusting the buffer concentration, and the distribution of the intermediates was monitored.

As seen in Figure 3, conversion of TCE to *cis*-DCE, VC, and ethene was strongly dependent on the pH of the recirculating liquid. A gradual decrease in the conversion to *cis*-DCE and an increase in the conversion to ethene were observed as the pH was lowered from 8.3 to 7.4. A much sharper change was observed at near neutral pH with the optimum conversion to ethene observed at pH 6.85. At pHs lower than 6.85, the TCE RE decreased to below 90% with only marginal improvement in the conversion to ethene. The reactor was operated for 20 days at pH 6.85 thereafter

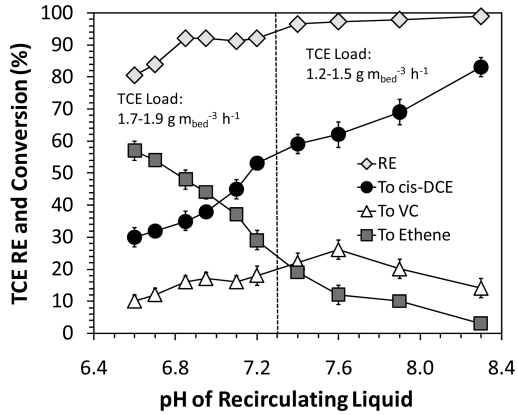

FIGURE 3. Effect of recirculating liquid pH on the TCE removal efficiency (RE) and the distribution of intermediates of TCE biological reduction. The error bars show standard deviations in pseudo steady-state conversions over 3−4 days.

and reached a steady state with greater than 90% TCE removed and conversion to ethene of ∼50%.

Later in the study (phase 10), the biotrickling filter was operated for 16 days at pHs of 6.85−6.91 and a faster liquid trickling rate and a higher TCE loading and performance were averaged (Table S4 in the Supporting Information). TCE conversion to ethene reached 63−67%, conversion of the removed TCE to *cis*-DCE and VC decreased to less than 16% and 20%, respectively, and the TCE elimination capacity reached 2.9 g m_{bed}^{-3} h^{-1}. The lower conversion to *cis*-DCE is likely because of enrichment on *cis*-DCE during several batch operation runs in the absence of TCE (see below). While these are high TCE removal rates, it was not possible to increase the conversion of TCE to ethene without significantly reducing the loading.

The complex behavior with respect to metabolite production raises interesting questions about the process culture. Although pH is a fundamental parameter, the literature is remarkably silent on the effect of pH on the activity of the reductive dehalogenase (RDase) enzymes identified in *Dehalococcoides* microorganisms, and the observation of the significant impact of pH on the distribution of intermediates should stimulate detailed study in this direction. Note that SDC-9 culture also contains a *Desulfitobacterium* strain. Microorganisms from *Desulfitobacterium* spp. are reported to dechlorinate TCE to *cis*-DCE but not further (*37, 38*). The RDase PceA, which has been documented for partial dechlorination of TCE by *Desulfitobacterium* microorganisms, is reported to have an optimum pH of close to 7.5 and does not lose activity up to pHs as high as 9.0 (*39*). This coupled with the observation that conversion beyond *cis*-DCE was poor at higher pHs indicates that these microorganisms may be the ones contributing primarily to TCE removal under alkaline conditions.

Biological Reduction rates of TCE, *cis*-DCE, and VC. The reductive dehalogenation kinetics of TCE, *cis*-DCE, and VC were further investigated after the biotrickling filter was modified to operate as a batch reactor (Figure S2 in the Supporting Information). This setup allowed rapidly determining reduction rates after small pulses of individual compounds are injected into the system. The apparent first-order rate constants were calculated as the slopes of the $\ln(C_0/C)$ vs time plots for batch reduction of each compound in the reactor (Figure S4 in the Supporting Information) after the initial phase of gas−liquid absorption. While the dehalogenation rates obtained using this method are apparent rates, their values relative to one another can be used for comparison.

First-order biological reduction rates for TCE, *cis*-DCE, and VC were evaluated as 2.05, 1.72, and 1.15 h^{-1}, respectively, and this indicates that VC is the slowest step in the dechlorination of TCE in the biotrickling filter. Thus, accumulation of VC rather than *cis*-DCE is expected during biotrickling filter operation. A possible explanation is that both TCE and *cis*-DCE are expected to compete for the active sites of the TceA enzyme in *Dehalococcoides* strains. However, because coupling TCE with hydrogen is more energetically favorable than coupling *cis*-DCE with hydrogen (*40*), *cis*-DCE reduction in the presence of TCE (i.e., during continuous operation) must have been slower in flow through experiments compared to the batch test where there was no competitive inhibition. Our observation of *cis*-DCE accumulation during continuous operation of the biotrickling filter is also consistent with the report that TCE inhibited *cis*-DCE dechlorination in microcosms containing mixed cultures with multiple *Dehalococcoides* strains (*41*).

Overall, this study demonstrates that anaerobic gas-phase treatment of TCE vapors in a biotrickling filter is possible. The complex behavior of the system with respect to the production of partially dechlorinated intermediates suggests that further process and/or culture optimization is needed to achieve complete conversion of TCE to ethene at a high rate. Once optimized, the process may provide new options for the cleanup of TCE contaminated sites.

Acknowledgments

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Supporting Information Available

Schematic diagram of the anaerobic biotrickling filter; schematic diagram of the anaerobic biotrickling filter converted to operate in batch mode; comparison of theoretical TCE reduction rate with the observed elimination rate during the startup of the anaerobic biotrickling filter; linear fits for the first-order biological reduction rate constants for TCE, *cis*-DCE, and VC using the batch data; composition of the modified RAM media; pressure drop across the anaerobic biotrickling filter bed during continuous operation; comparison of maximum TCE elimination capacities and longevity of operation between aerobic gas-phase bioreactors and this study; performance of biotrickling filter during phase 10; details about SDC-9 culture composition. This material is available free of charge via the Internet at http://pubs.acs.org.

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Supporting Information for

REDUCTIVE DEHALOGENATION OF TRICHLOROETHENE VAPORS IN AN ANAEROBIC BIOTRICKLING FILTER

Sudeep C. Popat, Marc A. Deshusses

Pages: 10
Figures: 4
Tables: 4

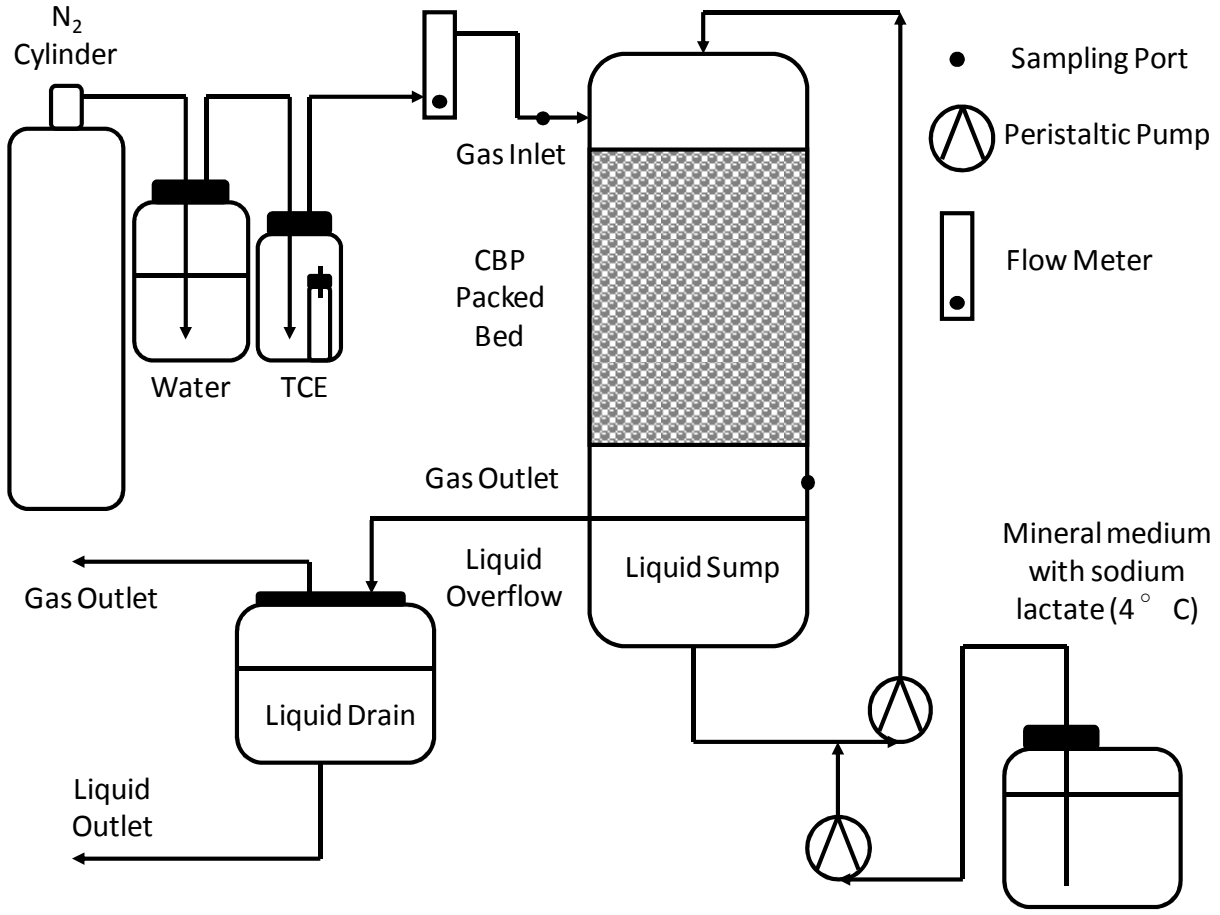

Figure S1. Schematic diagram of the anaerobic biotrickling filter.

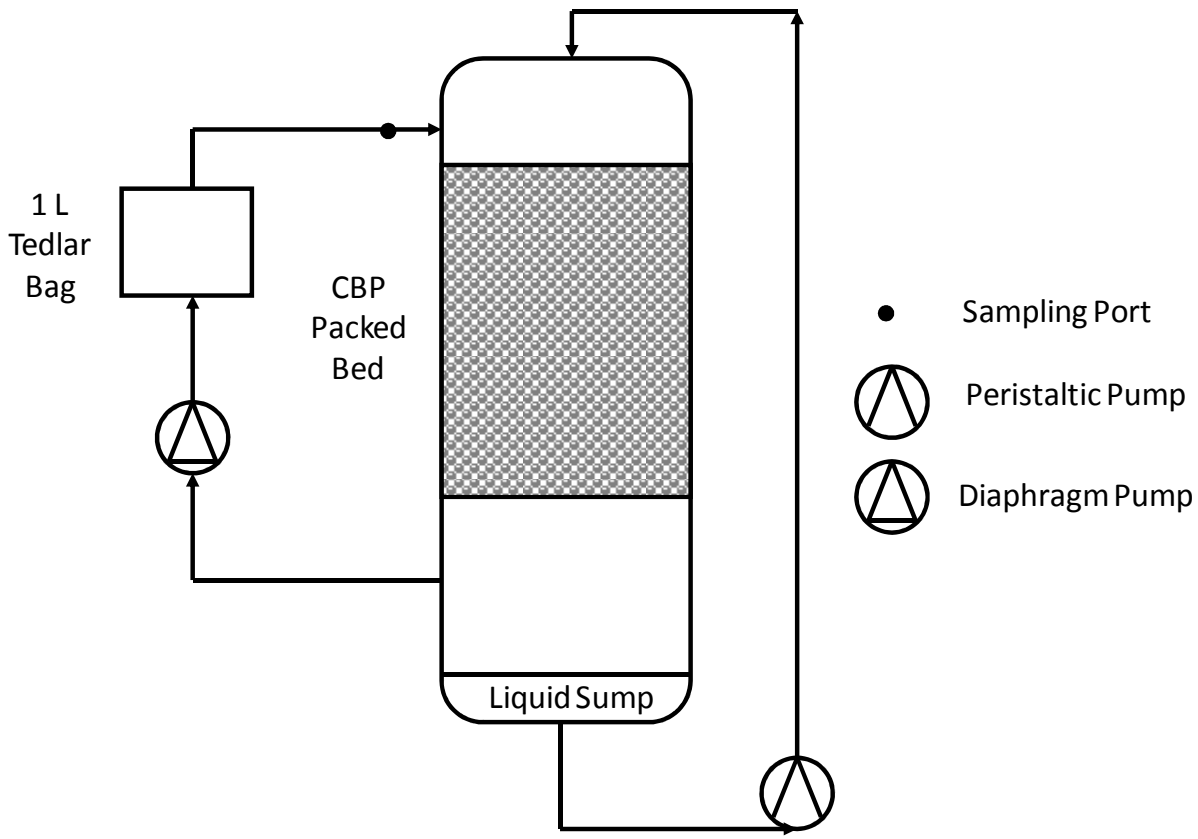

Figure S2. Schematic diagram of the anaerobic biotrickling filter modified to operate in batch mode.

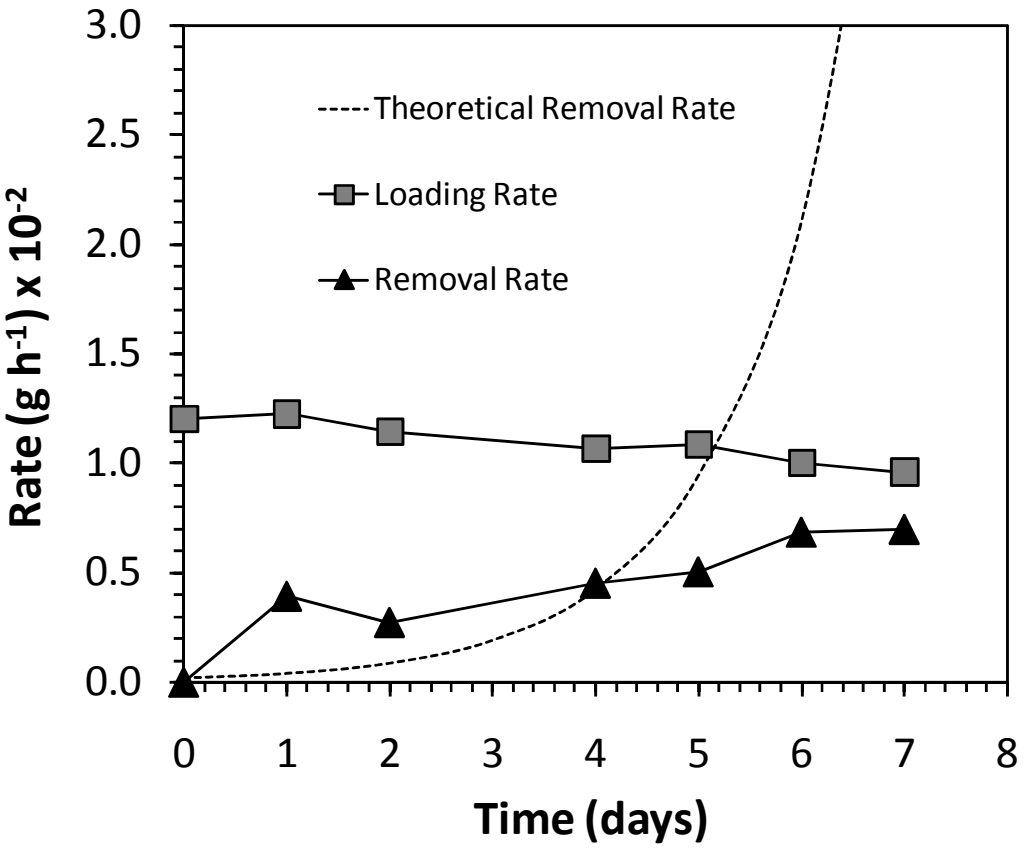

Figure S3. Comparison of the theoretical TCE degradation rate calculated assuming exponential growth of SDC-9 with the observed TCE elimination rate during the start-up of the anaerobic biotrickling filter.

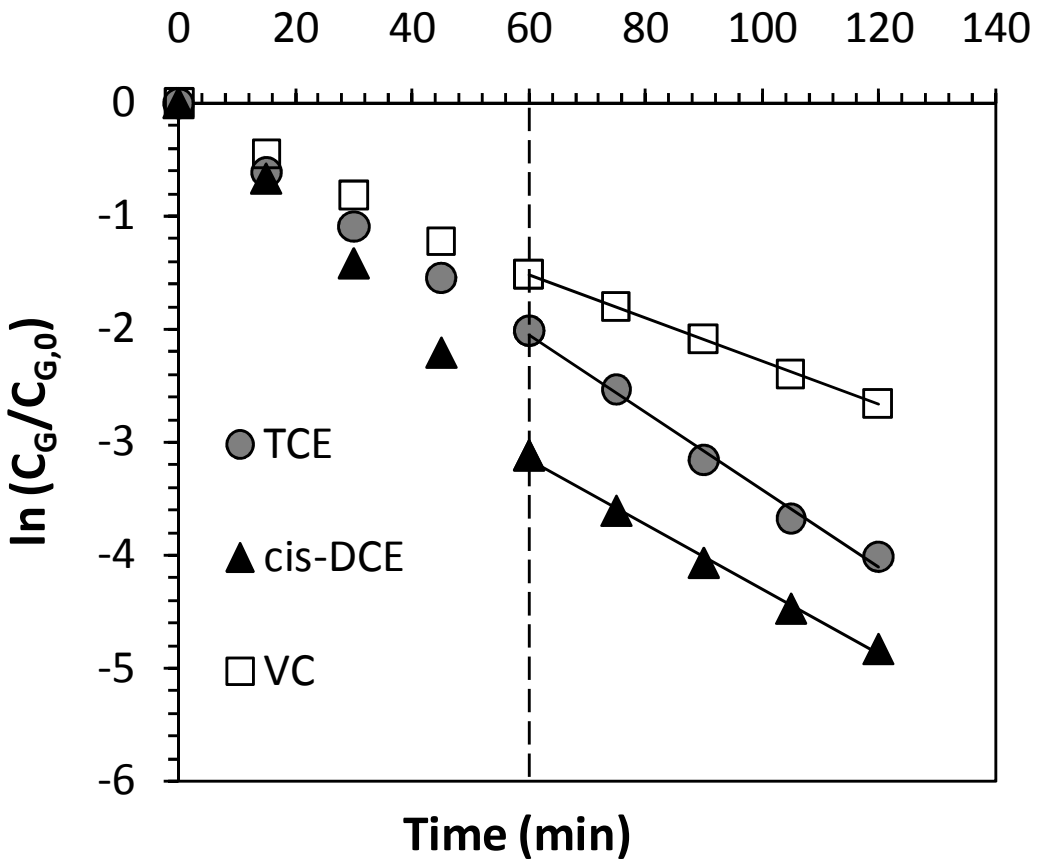

Figure S4. Linear fits for calculation of the first-order apparent biodegradation rate coefficients for TCE, cis-DCE and VC using the batch biotrickling filter data. During the first 60 min, absorption into the trickling liquid was the main removal mechanism, thus regression was only conducted on data between 60 and 120 min. All linear fits had R^2>0.99.

Table S1. Composition of modified RAM media (*S1*).

Compound	Concentration (g L^{-1})
KH_2PO_4	0.27-4.0*
K_2HPO_4	0.35-2.0*
NH_4Cl	0.53
$CaCl_2 \cdot 2H_2O$	0.075
$MgCl \cdot 6H_2O$	0.1
$FeCl_2 \cdot 4H_2O$	0.02
$NaHCO_3$	0.2-1.2
Trace elements**	5 mL L^{-1}
Yeast extract	1

*amount was varied to change pH (see text for details)

****Preparation of trace elements solution (*S2*):**
Bidest. H_2O: 3 L
HCl conc.: 33.8 mL
$FeCl_2 \cdot 4H_2O$: 7.5 g
Dissolve: H_3BO_3 0.3 g, $MnCl_2 \cdot 4H_2O$ 0.5 g, $CoCl_2 \cdot 6H_2O$ 0.6 g. $ZnCl_2$ 0.35 g, $NiCl_2 \cdot 6H_2O$ 0.125 g, $CuCl_2 \cdot 2H_2O$ 0.075 g, $NaMoO_4 \cdot 2H_2O$ 0.125 g. EDTA $Na_4(H_2O)_4$ 26 g
Fill to 4.8 L
Add NaOH until the pH is 4.2
Fill to 5 L

Table S2. Pressure drop across the anaerobic biotrickling filter bed during continuous operation.

Time of Operation (day)	**Pressure Drop (mm H_2O m_{bed}^{-1})**
5	1.8
21	6.1
36	7.6
67	8.3
79	14.2
97	8.4
114	9.6
156	9.8

The pressure drop across the reactor bed remained under 10 mm H_2O m_{bed}^{-1}, except for phase 6 (see Figure 1 and Table 1), when more lactate was added in the nutrient feed. The pressure drop increased from 8.3 mm H_2O m_{bed}^{-1} on day 67 to 14.2 mm H_2O m_{bed}^{-1} on day 79. This was likely due to an increased growth of fermentors from the additional lactate supply. A larger organic carbon (from lactate) elimination capacity (6-7 g m_{bed}^{-3} h^{-1} compared to 3-5 g m_{bed}^{-3} h^{-1} before) was noted for this phase. On day 80, the humidification chamber was removed from the inlet TCE line and the recirculation of the liquid sump was stopped for 6 hours, to remove excess biomass through bed drying. The bed was then shaken vigorously to cast off any loose dried biomass, before resuming normal operation. The effect of this disruption on the TCE removal efficiency was only nominal. The pressure drop across the bed decreased, and remained under 10 mm H_2O m_{bed}^{-1} for the rest of the operation.

Table S3. Comparison of maximum TCE elimination capacities and longevity of operation between aerobic gas-phase bioreactors and current study.

Type of Reactor*	**Primary Substrate**	**Maximum EC ($g\ m^{-3}\ h^{-1}$)**	**Stable Continuous Operation (days)****	**Ref.**
BTF	Peptone/glucose	0.4	NR	*S3*
CSTR	Toluene	3.1	NR	*S4*
Bubble column	Phenol	1.45	NR	*S5*
Membrane BF	Toluene	1.9	130	*S6*
ACBF	Phenol	4.3	190	*S7*
BFMM	Glucose	16.3***	80	*S8*
HFMB	Methanol	3.9	NR	*S9*
BTF	Propane	1.0	70	*S10*
CSTR+BTF	Phenol	0.7	50	*S11*
BF	Toluene	1.8	NR	*S12*
FEBR	Toluene	28	NR	*S13*
Anaerobic BTF	TCE	4.4	180	This study

*ACBF activated carbon biofilter, BF biofilter, BTF biotrickling filter, BFMM biofilter with mutant microorganisms, CSTR continuous stirred tank reactor, FEBR foamed emulsion bioreactor, HFMB hollow fiber membrane bioreactor

**NR not reported

***Rapid loss of performance was observed after the maximum EC was observed

Table S4. Summary of continuous operation of the anaerobic biotrickling filter for days 220-236 (phase 10).

EBRT	4.8 min
TCE inlet concentration	245-255 mg m^{-3}
TCE removal efficiency	90-92%
Recirculating liquid pH	6.85-6.91
Recirculating liquid ORP	82-94 mV
Recirculating liquid flow rate	68-72 mL min^{-1}
TCE load	3.1-3.2 g m_{bed}^{-3} h^{-1}
TCE elimination capacity	~2.9 g m_{bed}^{-3} h^{-1}
TCE conversion to cis-DCE	14-16%
TCE conversion to VC	~20%
TCE conversion to ethylene	63-67%

Composition of SDC-9 culture

Aceticlastic methanogens *(S14).* To qualitatively determine the presence of aceticlastic methanogens, SDC-9 was fed acetate only (3750 μM), and incubated for 87 hours. Analysis for acetate, hydrogen and methane after incubation period showed that acetate consumption was negligible and only 0.003 moles of methane were formed per mole of acetate fed, which was likely to be formed from hydrogen (as explained below). Only trace concentrations of hydrogen were detected (~3.3 μM). This experiment indicated that the original culture does not contain a significant amount of aceticlastic methanogens.

Hydrogenotrophic methanogens and acetogens *(S14).* To qualitatively determine the presence of hydrogenotrophic methanogens, SDC-9 was fed CO_2 and hydrogen (hydrogen was the limiting compound at a total of 90 μmoles initially in the gas phase), and incubated for 87 hours. Analysis for acetate, hydrogen and methane after incubation period showed that both acetate and methane formed significantly, with 0.15 moles of acetate formed per mole of H_2 fed, and 0.1 moles of methane formed per mole of H_2 fed. Given the fact that acetate-fed culture showed almost no methanogenesis (see above), it can be reasonably concluded from the significant methane formation in these microcosms that the methanogens present in the culture are hydrogenotrophic. Control microcosms (i.e. without CO_2+hydrogen) also showed similar acetate formation, suggesting that acetate was possibly produced from other sources and not just CO_2+hydrogen. Thus it was not possible deduce from these simple tests if the culture contains homoacetogens.

Dehalococcoides* sp. *(S14) qPCR analysis of SDC-9 when fed chlorinated ethenes revealed that the culture contains genes encoding for the PceA, TceA and VcrA dehalogenases, but not the BvcA dehalogenase. DGGE analysis of PCE-fed SDC-9 cultures revealed the presence of at least two *Dehalococcoides* strains in the culture (*S15*).

Other microorganisms *(S14).* DGGE analysis of PCE- and lactate-fed SDC-9 culture revealed the presence of a *Desulfovibrio* strain. *Desulfovibrio* spp., although documented to carry out reductive dechlorination of chlorophenols in the absence of sulfate (*S16*), are not known to dechlorinate PCE or TCE. However, certain *Desulfovibrio* strains (e.g. *Desulfovibiro vulgaris*) ferment lactate to acetate, CO_2 and hydrogen in the absence of sulfate. Thus, the detected *Desulfovibrio* strain in PCE- and lactate-fed SDC-9 was most likely involved in lactate fermentation. DGGE analysis of SDC-9 fed with 1,1,1-trichloroethane has shown the presence of a *Desulfitobacterium* strain. *Desulfitobacterium* spp. are reported to partially dechlorinate PCE and TCE to *cis*-DCE (*S17, S18*). However, no *Desulfitobacterium* strain was detected in PCE-grown cultures. Thus this strain could contribute to dechlorinating PCE and TCE at certain conditions. No *Dehalobacter* strain has been found in SDC-9 in any growth conditions.

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